

EXHIBIT I

INTRODUCTION AND SUMMARY

MK-933 is a member of the avermectin family of compounds which has significant antiparasitic activity. It and its separate constituents, L-638,709 (22,23-dihydro C-076 E₁a) and L-639,622 (22,23-dihydro C-076 B₁b), have been evaluated in a series of acute, subacute and teratogenicity tests which are summarized below and discussed in detail in the pages that follow. Except where stated to the contrary, the MK-933 tested was approximately an 80:20 mixture of dihydro B₁a and dihydro B₁b.

A. Acute Toxicity:

The acute oral toxicities of the components of MK-933, L-638,709 and L-639,622, were studied in the female mouse. The acute oral toxicity of MK-933 (L-640,471) was also studied in adult male and female mice, in young adult male and female rats and in infant rats.

The oral LD₅₀ values are tabulated below:

Compound-Lot No.	Species	Sex	Vehicle* Conc. %	Date of Study	LD ₅₀ (95% Fiducial Limits) mg/kg
L-638,709-00R04	Mouse	F	0.8	8/9/77	87.2 (39.4-198.0)
L-638,709-00R32	Mouse	F	0.8	2/12/79	31.7 (21.5-46.8)
L-639,622-00Z02	Mouse	F	0.8	4/10/78	56.6 (36.7-87.2)
L-639,622-00Z07	Mouse	F	0.8	2/12/79	27.6 (17.8-42.8)
L-640,471-00W01	Rat (I)	MF	varied	4/28/78	2.3 (1.5-3.4)
L-640,471-00W19	Mouse	F	0.8	1/18/79	24.6 (15.0-40.4)
L-640,471-00W19	Mouse	F	0.8	2/12/79	27.1 (18.6-39.4)
L-640,471-00W42 ⁴	Mouse	F	0.8	2/12/79	41.6 (28.1-61.5)
L-640,471-00W19	Mouse	F	0.8	2/23/79	40.0 (22.7-70.5)
L-640,471-00W19	Mouse	M	0.8	2/23/79	11.6 (4.7-29.0)
L-640,471-00W19	Rat ² (YA)	F	0.8	2/22/79	52.8 (39.2-71.0)
L-640,471-00W19	Rat ² (YA)	M	0.8	2/22/79	52.8 (39.2-71.0)
L-640,471-00W19	Rat ³ (YA)	F	0.8	3/13/79	44.3 (36.5-53.7)
L-640,471-00W19	Rat ³ (YA)	M	0.8	3/13/79	42.8 (37.2-49.2)

- * Sesame oil
- (I) Infant, 24-48 hours old
- (YA) Young animal
- (2) Sprague-Dawley, Camm
- (3) Charles River, CD
- (4) Lot 00W42 was an 84:16 mixture of L-638,709 and L-639,622 while lot 00W19 was an 80:20 mixture.

The variation in LD₅₀ values of MK-933 and its components may be due to the time interval between toxicity tests. When these compounds were tested concurrently in female mice (date, 2/12/79) there was no significant difference in the toxicity of the four compounds tested. (The Mantel-Haenszel Test was used to test difference in toxicity.)

The results also indicate that MK-933 (L-640,471) is significantly more toxic orally in the male mouse than in the female mouse. However, there was no significant sex-related difference in toxicity for the rat.

B. L-638,709 and L-639,622: Bacterial Mutagen Tests (Ames Test)

TT #77-8068 and TT #78-8105:

22,23-dihydro C-076 B₁a (L-638,709) and 22,23-dihydro C-076 B₁b (L-639,622) were tested separately for the ability to revert to histidine independence mutant strains of Salmonella typhimurium (TA1537, TA98, and TA100) that require histidine in order to grow. In addition, L-638,709 was tested with strain TA92 and L-639,622 was tested with strain TA1535. The tests were done with and without a metabolic activation system prepared from rat liver. L-638,709 was tested at compound concentrations up to 1000 mcg/plate and L-639,622 was tested at concentrations up to 2000 mcg/plate. Though the Standard Operating Procedure for our laboratory is to test compounds up to 2000 mcg/plate, insufficient amounts of L-638,709 were available to permit this. Neither compound produced significant increases in reversion to histidine prototrophy under any of the test conditions.

The positive control, 1-methyl-2(3a,4,5,6,7,7a-hexahydro-1,2-benzisoxazol-3-yl)-5 nitroimidazole, identified as MK-436, produced significant increases in revertants, particularly after metabolic activation with all of the water strains used.

C. L-638,709: Oral Range-Finding Studies in Pregnant Mice and Oral Teratogenic Study in Mice. TT #77-713-1, -2, -3 and TT #77-713-0:

In a range-finding study, L-638,709, the 22,23-dihydro derivative of C-076 B₁a, was administered orally as a solution in sesame oil to five groups of 5 pregnant mice each from Days 6 through 15 of gestation. The dosage levels were 0.4, 0.8, 1.6, 2.4, and 3.2 mg/kg/day, and an additional group of 5 mice served as a vehicle control.

Deaths, preceded by coma, occurred in mice at 2.4 mg/kg/day and 1.6 mg/kg/day. Tremors were also observed in one of the mice which died at 2.4 mg/kg/day. There was no mortality or physical sign of toxicity in mice at any other dosage level. Maternal weight gain in surviving mice was unaffected by treatment at any dosage level.

In two additional range-finding studies, L-638,709 was administered orally as a solution in propylene glycol to eight groups of 5 pregnant mice each from Days 6 to 15 of gestation. In one of the studies, four groups of mice were administered propylene glycol in a volume of 10 ml/kg and in the other study four groups of 5 mice each received propylene glycol at a volume of 1 ml/kg. The dosage levels of L-638,709 were 0.2, 0.4, 0.8, 1.6, and 2.4 mg/kg/day in each study, and two additional groups of 5 mice each served as vehicle controls and received propylene glycol in the same volume as the corresponding drug-treated groups. Propylene glycol at 10 ml/kg was not tolerated as there were deaths, lethargy, and weight losses in all control and drug-treated groups. With one exception, deaths among drug-treated mice were preceded in all cases by a comatose state with intermittent tremors. The one exception was a 0.2 mg/kg/day

mouse which was found dead on Day 18, and whose clinical appearance prior to death was similar to that of the control mice. The toxicity of the propylene glycol at 10 ml/kg does not allow a clear distinction between vehicle and drug-related effects, and any estimate of toxic threshold doses under these conditions would be unreliable. For this reason the second range-finding study was conducted in which the volume of propylene glycol was 1 ml/kg as indicated above. There were treatment-related deaths at 0.4, 0.8, 1.6, and 2.4 mg/kg/day. Tremors and coma were consistent findings in mice that died. There were no effects on average maternal weight gain among surviving mice at any treatment level. No adverse effect occurred in the 0.2 mg/kg/day group.

A teratology study was conducted in which L-638,709 was administered to four groups of 20 pregnant mice each from Days 6 through 15 of gestation. Dosage levels were 0.2, 0.4, 0.8, and 1.6 mg/kg/day and the drug was administered orally as a solution in sesame oil. Two additional groups of 20 mice each served as vehicle controls. No adverse maternal effect was observed at 0.2 mg/kg/day. There were treatment-related deaths at 0.4, 0.8, and 1.6 mg/kg/day. One mouse died in each of the 0.8 and 1.6 mg/kg/day dosage level groups and 1 additional mouse in each of these groups was sacrificed while aborting. Two mice in the 0.4 mg/kg/day dosage group were sacrificed, 1 while aborting and a second in a moribund condition. All mice that died or were sacrificed exhibited tremors and/or coma prior to being found dead, aborting, or being sacrificed as moribund. Teratogenicity, as evidenced by cleft palates in 10 fetuses from five litters, was seen at 1.6 mg/kg/day. No other treatment-related

external malformations were observed at any other dosage level. Visceral and skeletal examinations produced no further evidence of teratogenicity in any dosage group.

D. L-639,622: Oral Range-Finding Study in Pregnant Mice. TT #78-711-1:

22,23-dihydro C-076 (B_{1b}) (L-639,622) was administered orally in sesame oil to four groups of 10 pregnant mice each at dosage levels of 0.4, 0.8, 1.6, or 3.2 mg/kg/day from Days 6 through 15 of gestation. Another similar group of mice served as controls and received 10 ml/kg/day of sesame oil.

One mouse from each of the 3.2 mg/kg/day and 1.6 mg/kg/day groups showed ataxia, clonic convulsions, and were subsequently found dead. No other drug-related toxicity was observed. Average maternal body weight gains were unaffected by treatment at any dosage level.

E. L-639,622: Oral Teratogenic Study in Mice. TT #78-711-0:

The teratologic potential of 22,23-dihydro C-076 (B_{1b}) (L-639,622) was investigated in mice. 22,23-dihydro C-076 (B_{1b}) was administered orally to three groups of 20 pregnant mice from Days 6 through 15 of gestation at 0.4, 0.8, and 1.6 mg/kg/day. One additional group of 35 mice served as controls and received only the vehicle, sesame oil.

One female at 1.6 mg/kg/day became comatose and one 0.8 mg/kg/day female became prostrate after the first and fifth doses, respectively. Both animals were sacrificed. No other clinical signs of toxicity were observed in any treated group.

Average maternal body weight gains and reproductive status were unaffected by drug treatment.

Teratogenicity of 22,23-dihydro C-076 (B_{1b}) was revealed by external examination of fetuses; the frequency of cleft palate was increased in a dose-related fashion at 0.8 and 1.6 mg/kg/day. There was no external evidence of a teratogenic effect in fetuses at 0.4 mg/kg/day. Visceral and skeletal examinations revealed no further evidence of teratogenicity in any dosage group.

F. MK-933: Oral Reproduction Study in Rats. TT #78-710-0:

MK-933 was administered orally to three groups of 15 female rats each at dosage levels of 0.4, 0.8, and 1.6 mg/kg/day from 15 days before mating throughout gestation and lactation until Day 20 postpartum. Two additional groups of 15 females each served as controls and received the vehicle, sesame oil, in the same dosing regimen as the treated animals.

There was no mortality or clinical evidence of toxicity in females dosed with MK-933 at any treatment level. Average body weight gains in females given 0.4 mg/kg/day were not significantly different from the controls prior to breeding. Average weight was significantly increased ($P \leq 0.05$) among females at 0.8 and 1.6 mg/kg/day during the prebreeding period. Significant increases ($P \leq 0.05$) in average female body weight gain throughout the gestation period occurred at all dosage levels compared to controls. Average maternal weight gain during lactation was essentially similar to that of the controls.

MK-933 had no effect on the mating, reproductive status, average length of gestation, or postimplantation survival rate at any treatment level.

There was no treatment-related mortality or toxicity among pups from dams given 0.4 or 0.8 mg/kg/day. There were statistically significant ($P \leq 0.05$) treatment-related increases in mortality among pups in the 1.6 mg/kg/day dosage level group on Day 1 postpartum and from Days 7 to 14 postpartum. The increased mortality from Days 7 to 14 was primarily the result of 5 pups in one litter that died during this period. Prior to death these pups were observed to be hypothermic and to have no externally observable milk in the epigastric region, although 2 other pups in this litter were unaffected. Hypothermia was also observed on at least one occasion in pups from four other high-dose litters.

Average pup weights in the 0.4 mg/kg/day group were comparable to or slightly greater than controls throughout the lactation period. Average pup weights in the 0.8 and 1.6 mg/kg/day dosage level groups were significantly increased ($P \leq 0.05$) compared to control values throughout most of lactation. Development of the pups as measured by the appearance of ear opening, eye opening, incisor eruption, and hair growth was slightly but significantly ($P \leq 0.05$) accelerated in all treatment groups compared to controls and this is thought to be a reflection of the slightly greater increases in body weight of treated pups compared to the controls.

At the conclusion of this study, pups were randomly selected for continuation in a 14-week in utero toxicity study in rats (TT #78-037-0).

G. MK-933: 14-Week Toxicity Study in Rats following in utero Exposure. TT #78-037-0.

A three-month oral toxicity study with in utero exposure in rats at dosage levels of 0.4, 0.8, and 1.6 mg/kg/day was performed. At the start of the study, the rat pups were between 3 and 4 weeks of age; the males weighed 49 to 86 gm and the females weighed 43 to 77 gm.

Antemortem studies included detailed physical examinations five days a week, recording of body weights twice a week, and routine ophthalmoscopic, hematologic and biochemical studies in Drug Weeks 4, 8, and 13. Postmortem studies included routine necropsy examination of all rats including recording of weights of spleen, heart, kidneys, testes, liver, and brain and detailed microscopic examination of various tissues of all Control I and high dose rats. All tissues exhibiting gross lesions and liver, kidney, spleen, and bone and marrow from all rats were also examined microscopically.

No changes due to treatment occurred at 0.4 mg/kg/day.

There were no abnormal physical signs or mortality attributable to treatment. Also, there were no ocular abnormalities and no changes in the routine hematologic or biochemical parameters which were related to treatment.

Body weight gain in treated rats was not statistically significantly different from that of the controls.

The spleens of 4 rats (3 at 1.6 mg/kg/day and 1 at 0.8 mg/kg/day) were enlarged. On microscopic examination there were varying degrees of congestion in the red pulp and varying amounts of extramedullary hematopoiesis. These rats also showed iron-positive pigment in the renal tubular epithelium. One of these rats showed yellow-brown pigment in the Kupffer cells. These changes suggest a possible drug-related intravascular hemolysis, but the mechanism is not known. There was reactive hyperplasia of the bone marrow in these rats considered related to intravascular hemolysis. One of the rats (1.6 mg/kg/day) with an enlarged spleen also had a moderate degree of hepatocytic cytoplasmic vacuolation. However, very slight or slight vacuolation of the liver cell cytoplasm was observed in 6 other rats (2 each in the control, 0.8 and 1.6 mg/kg/day groups); thus, the relationship of the greater degree of this change to other changes suggesting intravascular hemolysis is uncertain. There were no other microscopic changes related to treatment at these dosage levels.

H. MK-933: Fourteen-Week Oral Toxicity Study in Dogs. TT #78-038-0:

Twenty male and 20 female beagles were selected for a 14-week study of MK-933. At initiation of the study the dogs were 39 to 43 weeks of age and ranged in body weight from 6.2 to 12.1 kg. The compound in sesame oil was administered by gavage at doses of 0.5, 1.0, or 2.0 mg/kg/day for 94 to 95 consecutive days to groups of 8 dogs each (4 males and 4 females). Two similar groups of animals received either deionized water or sesame oil alone and served as controls. All dogs

were examined five days a week for physical signs of abnormality (with less detailed examinations on the weekends) and daily for mortality. The animals were weighed twice a week during the study. Hematologic and serum biochemical studies and urinalyses were performed in Drug Weeks 4, 8, and 12. Ophthalmologic examinations were conducted in Drug Weeks 3, 7, and 12, while electrocardiograms were taken in Drug Weeks 5, 9, and 13. All surviving animals were killed and necropsied in Drug Week 14.

Mydriasis and slight weight loss were observed at doses of 1.0 and 2.0 mg/kg/day. Four dogs in the 2.0 mg/kg/day group developed tremors, ataxia, anorexia, and dehydration and were sacrificed prior to the scheduled necropsy. No gross abnormalities were observed.

Postmortem studies conducted on all dogs disclosed no treatment-related gross, microscopic or organ weight changes except for small amounts of agonal gastrointestinal congestion and/or hemorrhage seen in 2 of 4 dogs in the 2.0 mg/kg/day group that were killed prior to termination of the study.

SAFETY EVALUATION:

MK-933 is highly toxic but is not mutagenic as assessed by the Ames test.

L-638,709 was maternotoxic for pregnant mice at doses of 0.4 mg/kg/day and higher and for L-639,622 a minimum toxic dose under similar conditions was 0.8 mg/kg/day. Each compound induced cleft palates in mice, L-638,709 at doses of 1.6 mg/kg/day and L-639,622 at 0.8 mg/kg/day or greater. No

teratogenic effects were seen at or below doses of 0.8 or 0.4 mg/kg/day, respectively.

In a reproduction study in rats as well as in acute studies, it was demonstrated that neonates are significantly more susceptible to the toxic effects of MK-933. The LD₅₀ for infant rats is approximately 10-fold less than that of adults. In the reproduction study overt toxicity, i.e., increased neonatal mortality occurred at 1.6 mg/kg/day. While in the companion 14-week toxicity study in which rats derived from the reproduction study were given MK-933 at doses up to 1.6 mg/kg/day, no mortality occurred.

In the 14-week oral study in rats, no treatment-related effects were observed at a dose of 0.4 mg/kg/day. At doses of 0.8 and 1.6 mg/kg/day, enlarged spleens resulting from congestion and extramedullary hematopoiesis occurred in a few rats. This was accompanied by the accumulation of iron-positive pigment in the renal tubular epithelium and hyperplasia of the bone marrow.

In a 14-week oral study in dogs, no treatment-related effects were observed in animals given 0.5 mg/kg/day. Dogs given 1.0 and 2.0 mg/kg/day developed mydriasis and lost a small amount of weight. Four of 8 dogs given 2 mg/kg/day developed tremors, ataxia, anorexia and became dehydrated. These dogs were killed prior to termination of the study. Agonal gastrointestinal hemorrhage and/or congestion was observed in 2 of these dogs. No other treatment-related histologic change was observed in any dog.

These studies suggest that MK-933 and its separate components are qualitatively and quantitatively, within the limits of the test systems, similar with respect to toxicity.

Radioactive studies of MK-933 in cattle, sheep and swine (R.N. 189, 190, 192, and 194) indicate a maximum mean residue in fat, the "target" tissue, at 28 days off drug, of 36 ppb. Given the no-effect levels mentioned above, this should provide quite an adequate margin of safety for the proposed clinical use of MK-933 in these species.